Abstract

Proteins, participating in a myriad of biological function, are at the core of all cellular activities occurring within living organisms. Therapeutic proteins, hence constitute a major part of the pharmaceutical industry. The glycoprotein hormones follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH) and human chorionic gonadotropin (CG) regulate various reproductive and metabolic functions in humans and hence have high therapeutic potentials. The increasing demand of recombinant proteins for therapeutic uses drives the development of better expression systems.

The methylotrophic yeast *Pichia pastoris*, has been termed as an industrial workhorse for heterologous protein expression. However, the N-glycosyalation in yeast is of the high mannose type, resulting in a reduced serum half-life of the recombinant proteins. In the current work, we have re-engineered the Pichia N-glycosylation pathway to mimic the human type of N-glycosylation. Towards this end, we abolished the yeast native N-glycosylation and introduced enzymes from various eukaryotic sources into the system. These modifications resulted in the conversion of the yeast $Man_{9\text{-}20}GlcNAc_2$ glycan structure to a more human like $GlcNAc_2Man_3GlcNAc_2$ form on over 70 % of the heterologous expressed proteins.

In order to demonstrate the application of these strains as efficient protein expression hosts, the glycoengineerd Pichia was used for large scale expression of the glycoprotein hormones, hCG and FSH. The purified recombinant hormones were found to have binding affinities and structure similar to that of the natural hormones. These recombinant hormones were also able to elicit over two fold responses in animal models compared to buffer controls and the activity was comparable to the natural couterparts. Thus, we report the generation of a glycoengineered *Pichia pastoris*, which can be considered as a serious contender for the expression of glycosylated proteins of therapeutic importance.